REMARKS

Claims 1-4, 36-49 are pending in the above-identified application.

Support for claims 1, 36-39 can be found on pages 9-10 (including Table 2); pages 11-12 (including Table 4); and page 31, line 3 to page 32, line 28.

Support for claims 40-44 can be found on page 6, line 32 to page 7, line 2; pages 9-10 (including Table 2); pages 11-12 (including Table 4); page 20, lines 19-31; page 31, line 3 to page 32, line 28; page 39, lines 6-16; and page 46, line 26 to page 47, line 5. Antibodies in general are described on page 39, line 6 to page 46, line 9.

Support for claim 45 can be found on page 35, lines 14-17.

Support for claim 46 can be found on pages 35-36.

Support for claim 47 can be found in Table C and pages 35-36.

Support for claims 48-49 can be found on page 7, lines 13-20.

There is no status for a claim 32 because no claim 32 was originally filed.

Cancer claims over half a million individuals a year in the US alone and is the second leading cause of death cit_bf(American Cancer Society, 2002)cit_af ref_bf(American Cancer Society 2002 ref_num17)ref_af. Despite the many advances in cancer research, diagnosis and therapy, much remains to be learned before this killer can be tamed.

One approach that yields not only information about cancer cells, but also tools for cancer treatment and diagnosis, is differential expression analyses in appropriate model systems. The elucidation of molecular events that correlate with cellular transformation would enable early detection, the prescription of effective treatment and provide therapeutic targets (page 1, lines 23-26; page 4, lines 20-25; page 15, lines 4-14).

Oncogenes, such as *Wnt-1*, are genes that when mis-regulated are linked to cancer; these genes usually encode polypeptides that control cell growth or its regulation (page 1, lines 7-11). In the case of Wnt, family members are cysteine-rich, glycosylated signaling proteins that mediate diverse developmental processes, such as the control of cell proliferation, adhesion, cell polarity, and the establishment of cell fates (page 1, lines 7-11). Components of the Wnt signaling pathway have been linked to tumorigenesis in familial and sporadic colon carcinomas, breast cancer, and melanoma (page 1, lines 7-11; 23-32).

Wnt-1 itself was shown to be an oncogene in mouse mammary tumors, but this gene was not significantly up-regulated in most human breast carcinoma cells (Nusse and Varmus, 1992; Tsukamoto *et al.*, 1988), seemingly contradicting the mouse results. The plain correlation of *Wnt-1* up-regulated expression with human breast carcinoma, however, is too simplified: molecular targets in the Wnt-1 signaling pathway are more likely to be oncogenes (Brown 2001)

The inventors have identified an important downstream cellular component of the Wnt-1 signaling pathway in mammary cells transformed by *Wnt-1*, solving the problem of the identification of down-stream Wnt-1 targets. Such a molecule is useful in the diagnosis and treatment of carcinomas, such as breast carcinomas (page 1, lines 23-26; page 4, lines 20-25; page 15, lines 4-14).

To identify downstream targets in the Wnt signaling pathway that are relevant to the transformed cell phenotype, the inventors looked at gene expression in *Wnt-1*-expressing C57MG mouse mammary epithelial cells compared to the gene expression pattern found in normal C57MG and in *Wnt-4*-expressing C57MG cells (page 4, line 26 to page 5, line 2). *Wnt-1*, when expressed in C57MG cells, induces a partially transformed phenotype that is characterized by a loss of epithelial characteristics and contact inhibition (Brown *et al.*, 1986; Wong *et al.*, 1994). *Wnt-4*, however, does not induce tumors and autocrine cellular transformation. These cells, along with control, non-*Wnt-1*-expressing but *Wnt-4*-expressing C57MG cells, present an ideal system to identify those genes that contribute to the transformed phenotype (page 81, lines 8-9). /Mouse *STRA6* (*mSTRA6*) was up-regulated eleven-fold in *Wnt-1*-transformed cells when compared to the *Wnt-4* expressing control cells (page 82, lines 4-5).

The human STRA6-like (hSTRA6) polypeptide exhibits strong homologies and sequence identity to the mSTRA6 protein (page 12, lines 2-14; Table 5). These molecules share more than just homology, but also structural and cell biological features. As shown by hydrophobicity plots (Fig. 1), both mSTRA6 and hSTRA6 have 7-8 membrane spanning domains (page 14, lines 11-14). PSORT analysis indicates that both proteins localize to the cell membrane; and in fact, mSTRA6 does indeed localize there (Bouillet *et al.*, 1997; page 2, lines 5-7), as does hSTRA6 (Szeto *et al.*, 2001, p. 4202, 2nd column, 3rd paragraph and Fig. 8D).

REQUEST FOR RECONSIDERATION

Applicants respectfully traverse the rejections of claims 1-4 under 35 USC §§ 101 and 112. Art published after the filing of the application further supports the assertion that hSTRA6 plays important roles in cancers driven by *Wnt-1* (Szeto *et al.*, 2001; Tice *et al.*, 2002). From this, one utility for hSTRA6 is that of a diagnostic marker for cancers, such as colon and breast cancer and melanoma.

The asserted diagnostic utility for hSTRA6 is (1) credible, (2) specific and (3) substantial. The utility is credible: mouse (mSTRA6; GenBank AF062476 (represented as a translated polypeptide in SEQ ID NO:7)) is differentially expressed in Wnt-1-transformed cells, indicating that hSTRA6 is a target in the Wnt-1 signaling pathway and which, when perturbed, results in oncogenesis. The utility of the invention is also specific: the specificity of the discovered sequence was built into the experimental design--hSTRA6 differential regulation relates to cellular transformation. The utility is substantial: many people in the United States alone die from cancer.

An assertion of utility is credible unless the logic underlying the assertion is seriously flawed, or the facts upon which the assertion is based are inconsistent with the logic underlying the assertion (*Revised interim guidelines training materials*, no date given). Brown (2001), Smalley and Dale (2001), Moon *et al.* (2002), Szeto *et al.* (2001) and Tice *et al.* (2002) all support the asserted utility.

A utility for cancer diagnosis and treatment for downstream Wnt signaling targets has been asserted in the literature, although the full the array of molecules has not yet been completely defined. This invention adds a significant novel molecule to this array. The available literature teaches a utility for Wnt signaling targets, among which hSTRA6 is a member. Downstream signaling events initiated by Wnt ligand binding are implicated in breast cancer (Brown, 2001). In fact, "mutations in proteins involved in Wnt signal transduction have been demonstrated in human tumors[,] including colon, liver, skin, uterine, prostate, and ovarian cancers (*e.g.*, APC and β-catenin proteins)" (p. 37, 2nd column, final 4 lines of first paragraph; cit_bfSmalley and Dale (2001)cit_af ref_bf(Smalley, M. J. 2001 ref_num19)ref_af). Thus aberrant Wnt signaling, resulting in mis-regulated downstream targets, leads to oncogenesis. Furthermore, downstream Wnt signaling targets have been specifically asserted as being useful

in therapy (see Abstract of cit_bfMoon *et al.*, 2002cit_af ref_bf(Moon , R. T. 2002 ref_num21)ref_af). The utilities of hSTRA6 not only comprise the treatment and detection breast cancer, but also colon cancer and melanomas.

A molecule with substantial identity to the STRA6-like polypeptide of the present invention, *STRA6*, was found in the same type of study. Szeto *et al.* (2001) teach that (1) *STRA6* is up-regulated (as is *STRA6-like*; *e.g.*, page 82, lines 4-7 of the specification); (2) *STRA6* expression is synergistically up-regulated with retinoic acid (RA; *mSTRA6* having been shown previously to be similarly up-regulated by RA); and (3) stimulation of human colorectal cancer cell lines with RA also up-regulates *hSTRA6*, resulting in an accumulation of the protein at the cell membrane. Tice *et al.* (2002) confirm and expand these findings for *STRA6*. RA stimulation of *Wnt-1*-expressing C57MG cells greatly up-regulates *STRA6* expression. Instead of using GeneCalling to identify differentially expressed sequences in these experiments, Tice *et al.* (2002) probed an array of 12,000 oligonucleotides on an Affymetrix chip; this experiment independently verified the findings Szeto *et al.* (2001). When *Wnt-1*-expressing C57MG cells and human colon tumor xenografts were implanted in mice, RA greatly stimulated *STRA6* expression in the resulting tumors, but not in surrounding wild-type tissues.

STRA6-like strongly resembles *STRA6*, both in structure and regulation. *STRA6-like* exhibits similar up-regulation (10.9-fold (Quantitative-PCR), 11-fold (QEA); page 82, lines 4-7) as *STRA6*, has high homology with *STRA6*, and was found in the same cells as *STRA6*. Therefore, Applicants submit that *STRA6-like* will likely have the same utilities as *STRA6*. Indeed, even though one of the primers used in the initial STRA6 studies of Szeto *et al.* (2001) does not match *STRA6-like*, the probe they used would have recognized both *STRA6* and *STRA6-like*.

The invention meets the criteria of 35 USC § 101. Because the utility requirements are met, one of skill in the art would know how to use the invention. Applicants respectfully request withdrawal of the rejections made under 35 USC § 101 and 35 USC § 112, 1st paragraph.

Applicants respectfully traverse the rejection of the claims under 35 USC § 112, 1st paragraph. Applicants were in full possession of the claimed invention at the time of filing because the sequences of the polypeptides are given in the specification and useful variants are

described. Furthermore, well-known principles guide those of skill in the art to produce functional variants.

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented (MPEP § 2163.04, p.2100-168 (August 2001); *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971)). Compliance with the written description requirement does not require an applicant to describe exactly the subject matter claimed; rather, the description must allow one of ordinary skill in the art to recognize that the applicant has invented what is claimed (MPEP § 2163.02, p. 2100-167 (August 2001); *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989); *Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991)). A written description of an invention involving a chemical genus requires a precise definition, such as by structure, formula ... of the claimed subject matter sufficient to distinguish it from other materials (*Univ. of California v. Eli Lilly and Co.*, 43 USPQ2d 1398, 1405 (Fed. Cir. 1997)). Since one skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass, such a formula is normally an adequate description of the claimed invention. *Id.* at 1406.

The Applicants have provided such a formula. The sequence of hSTRA6 is given in Tables 2 (SEQ ID NO:2) and 4 (SEQ ID NO:4). Furthermore, an art-accepted method for calculating sequence identity has also been provided (page 31, line 3 to page 32, line 28). The specification teaches variants of SEQ ID NOs:2 and 4 (page 35, line 5 through page 36; chimeric and fusion polypeptides comprising the polypeptide sequence of SEQ ID NOs:2 and 4). Variants that maintain immunologic activity are also described formulaically (page 20, lines 28-31). Finally, conservative amino acid substitutions are described on page 24, line 28 to page 26, line 18.

The Office has extensively cited Bowie *et al.* (1990) to support its position. Bowie *et al.* note that while the problem of predicting protein structure from primary sequence, as well as function, can be complex (page 1306, column 1, they also note that certain general principles have been established. These principles can be applied to STRA6-like polypeptide variants, which are consistent with the teachings of the specification. These principles fall in the following categories:

(1) The nature of surface *vs.* buried residues in the folded protein;

(2) The hydrophobic nature of core sequences;

(3) The interchangeable nature of surface sites; and

(4) The roles of variant residues in related sequences.

Residues that are buried in the protein require non-polar side chains (p. 1306, column 2, third full paragraph); while surface residue side chains are much more interchangeable since few features of side chains are conserved (*ibid.*). This principle is elegantly illustrated in Figure 1, where those residues that are highly conserved in λ repressor are known to be buried residues (5 of 6), while those sites that can tolerate many different substitutions are known to be on the surface.

Core sequences, because of their importance in folding and stability--which are driven by the hydrophobic effect--require almost exclusively hydrophobic and neutral residues (p. 1307, column 1, first full paragraph). While core sequences are limited to these classes of amino acids, they are mostly interchangeable with each other because the hydrophobic effect does not depend on residue pairing (*ibid.*). Even within the core, the factors of hydrophobicity, packing volume and steric compatibility are not equally "informative" (p. 1307, second column, first full paragraph). While physically, these factors are all important, the factor of hydrophobicity of a sequence, rather than the factor of total side chain volume, predicts more about the side chain's acceptability as a member of the core, while the factor of steric compatibility falls midway between the two (p. 1308, column 1, top).

Each surface site can accommodate many side-chain substitutions, although most proteins can tolerate only a limited number of hydrophobic substitutions overall (p. 1308, column 1, first full paragraph). This principle is due to the assumption that large patches of hydrophobic surface residues would lead to aggregation (*ibid.*), which would presumably inhibit function.

Finally, using a set of related sequences, such as available for SEQ ID NOs:2 and 4, can guide amino acid substitution to make protein variants. By aligning the sequences of a family, such as SEQ ID NO:4 against *STRA6* isoform 2 (GenBank Accession AAK30290), conserved residues are likely to be important in folding and function. Those positions that are most variant are "almost certain" to be on the protein surface (p. 1308, column 1, second full paragraph), while positions that remain hydrophobic between the different proteins are most likely buried in the protein (*ibid.*).

Finally, these principles, combined with structure-predicting algorithms, can guide one of skill in the art in making functional variant polypeptides.

Applicants submit that the written description requirement has been met. Withdrawal of this ground of rejection is respectfully requested.

Rejection of the claims under 35 USC § 102(e) has been obviated by amendment.

References provided herewith

Brown, A.M. (2001) Wnt signaling in breast cancer: have we come full circle?. *Breast Cancer Res* 3, 351-355.

Moon, R.T., Bowerman, B., Boutros, M. and Perrimon, N. (2002) The promise and perils of Wnt signaling through beta-catenin. *Science* 296, 1644-1646.

Smalley, M.J. and Dale, T.C. (2001) Wnt signaling and mammary tumorigenesis. *J Mammary Gland Biol Neoplasia* 6, 37-52.

Szeto, W., W Jiang, D.A. Tice, B. Rubinfeld, P.G. Hollingshead, S.E. Fong, D.L. Dugger, T. Pham, D.G. Yansura, T.A. Wong, J.C. Grimaldi, R.T. Corpuz, J.S. Singh, G.D. Frantz, B. Devaux, C.W. Crowley, R.H. Schwall, D.A. Eberhard, L. Rastelli, P. Polakis, and D. Pennica. 2001. Overexpression of the retinoic acid-responsive gene *Stra6* in human cancers and its synergistic induction by Wnt-1 and retinoic acid. *Cancer Res.* 61:4197-4205.

Tice, D.A., W. Szeto, I. Soloviev, B. Rubinfeld, S.E. Fong, D.L. Dugger, J. Winer, P.M. Williams, D. Wieand, V. Smith, R.H. Schwall, D. Pennica and P. Polakis. 2002. Synergistic induction of tumor antigens by Wnt-1 signaling and retinoic acid revealed by gene expression profiling. *J. Biol. Chem.* 277:14329-14335.

Other cited references

American Cancer Society. (2002) *Cancer Facts and Figures*. Atlanta, GA: American Cancer Society, Inc..

Bowie, J.U., J.F. Reidhaar-Olson, W.A. Lim, and R.T. Sauer. 1990. Deciphering the message in protein sequences: Tolerance to amino acid substitutions. *Science* 247:1306-1310.

Brown, A.M., R.S. Wildin, T.J. Pendergast, and H.E. Varmus. 1986. A retrovirus vector expressing the putative mammary oncogene int-1 causes partial transformation of a mammary epithelial cell line. *Cell* 46:1001-1009.

Nusse, R. and Varmus, H.E. (1992) Wnt genes. *Cell* 69, 1073-1087.

Tsukamoto, A.S., Grosschedl, R., Guzman, R.C., Parslow, T. and Varmus, H.E. (1988) Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. *Cell* 55, 619-625.1.

Wong, G.T., B.J. Gavin, and A.P. McMahon. 1994. Differential transformation of mammary epithelial cells by Wnt genes. *Mol. Cell. Biol.* 14:6278-6286.

CONCLUSION

Reconsideration and withdrawal of all claim rejections is respectfully requested. Applicants believe that all claims in the present application are in condition for allowance.

Should the Examiner have any questions, or would like to discuss any matters in connection with the present application, the Examiner is invited to contact the undersigned at (312) 876-8936.

Respectfully submitted,

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Gregory M. Zinkl, Ph.D.
Registration No. 48,492
Agent for Applicants
SONNENSCHEIN NATH & ROSENTHAL LLP
P.O. Box 061080
Wacker Drive Station, Sears Tower
Chicago, Illinois 60606-1080
(312) 876-8000

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